3

Inheritance of Melanophore Patterns and Sex Determination in the Montezuma Swordtail, *Xiphophorus montezumae cortezi* Rosen

Klaus D. Kallman[1]

(Figures 1-10)

The inheritance of four melanophore patterns was studied in the teleost *Xiphophorus montezumae cortezi* endemic to parts of the Rio Panuco system, Mexico. Three of them, At (atromaculatus), Cam (carbomaculatus), and Sc (spotted caudal) are composed of macromelanophores and the fourth one, Cb (caudal blot), of micromelanophores. The patterns are controlled by four loci that are not linked and not associated with sex. No abnormal sex ratios were obtained. At, Cam, and Cb are dominant, but *Sc* exhibits incomplete penetrance in the homozygous and heterozygous conditions. The penetrance of *Sc* in an inbred laboratory stock is about 88 percent; in hybrids between this stock and wild fish, the penetrance of *Sc* is only 30 percent. The frequency of the *Sc* factor in the population of the Rio Axtla has been estimated to be about 59 percent. Within the inbred stock, the expression of *Sc* may vary from a small elongate streak in the caudal fin to large melanomas that eventually destroy it. The melanoma may spread into the caudal peduncle. No fish with melanoma have been seen in preserved collections of *X. m. cortezi* or in hybrids between the inbred stock and wild fish. All the major populations studied are polymorphic for the four patterns, although there may be significant differences in their frequencies. The situation in *X. m. cortezi*, where the macromelanophore patterns are controlled by three unlinked loci, contrasts with the one present in *X. maculatus* and *X. variatus*, where the patterns are controlled by the same gene or supergene.

Introduction

The genus *Xiphophorus* provides excellent material for the study of evolutionary processes at various taxonomic levels, because a variety of characters that can be analyzed genetically are present in related forms (e.g. *pigment patterns:* Anders and Klinke, 1965; Atz, 1962; Gordon, 1951; Kallman and Atz, 1966; Zander, 1962, 1969; sex determination: Dzwillo und Zander, 1967; Gordon, 1952; Kallman, 1965, 1968, 1970a; Kosswig and Öktay, 1955; Peters, 1964; *behavior:* Clark, Aronson and Gordon, 1954; Franck, 1964, 1970; *gonopodial traits:* Gordon and Rosen, 1951; Sengun, 1949). Nine of the 17 described species or subspecies are polymorphic for one or more macromelanophore patterns (Kallman and Atz, 1966; Rosen and Kallman, 1969). Best studied are those of *X. maculatus*. A very large number of crosses has shown that they are controlled by sex-linked factors that are members of the macromelanophore locus. However, at least two cases among several thousand offspring are known in which two different macromelanophore genes have become linked to each other (MacIntyre, 1961; Kallman and Schreibman, 1971). There is also some evidence that a modifier is adjacent to the pigment gene that regulates its expression. Thus the macromelanophore patterns are controlled by a complex locus.

Another interesting fact that has emerged from comparative genetic studies is that identical patterns in different populations of the same species have a different genetic basis (caused by different alleles at the major pigment locus

[1]Genetics Laboratory, Osborn Laboratories of Marine Sciences, New York Zoological Society, Brooklyn, N.Y. 11224.

interacting with population-specific modifiers) (Kallman, 1970b).

All available evidence indicates that no macromelanophore factor is present in more than one species, but admittedly the patterns of species other than *maculatus* have been poorly studied. The few crosses made with *X. variatus* and *X. milleri* indicate that their macromelanophore patterns are also under the control of sex-linked genes. The number of progeny raised are too small, however, to determine whether their macromelanophore factors are pseudoalleles also. Because the gonosomes of *maculatus, variatus* and *milleri* are homologous, Kallman and Atz (1966) suggested that all three species arose from an ancestral form (XX ♀♀— XY ♂♂) with a sex-linked macromelanophore locus. The two spotted patterns of *X. hellerii*, Db^1 and Db^2, are not associated with sex; no experiment has yet been performed that would test whether they are alleles. The chromosome that carries Db^1 is not homologous to the sex chromosomes of *maculatus* (Gordon, 1958; Kallman and Atz, 1966). Atz (1962), Kallman and Atz (1966), and Zander (1965) pointed out that in *X. montezumae cortezi* the two known macromelanophore patterns, *Sc* (spotted-caudal) and At (atromaculatus) were caused by different genes that were not linked. There is some evidence that *Sc* is located on a chromosome that is homologous to the sex chromosome of *maculatus* (Breider and Mombour, 1949; see also comment by Kallman and Atz, 1966).

During a recent field trip to the Rio Moctezuma, San Luis Potosi, Mexico, some *X. m. cortezi* were collected with macromelanophore spotting that differed from At in consisting of fewer but larger markings on the flank. The present report is an account of the genetic basis of the new pattern and also of caudal-blot, Cb, the only known tailspot pattern for which *X. m. cortezi* is polymorphic (Gordon, 1940; Kallman and Atz, 1966; Rosen, 1960).

Material and Methods

The Montezuma swordtail, *Xiphophorus montezumae* Jordan and Snyder, is endemic to the Rio Panuco-Rio Tamesi drainage. Two subspecies are recognized (Rosen, 1960). As far as is known, *X. m. montezumae* inhabits the headwater streams of the Rio Tamesi (Rio Frio, Rio Sabinas, but not Rio Guayalejo) and the northern and western tributaries (Rio Salto de Agua, Rio Verde) of the Rio Panuco (Rosen, 1960, Darnell, 1962) while *X. m. cortezi* is restricted to the headwaters of the Rio Moctezuma and Rio Tempoal (Rio Calaboza) that drain into the Rio Panuco from the south. With the exception of seven fish (four from Rio Calaboza system and three from the rather dubious location "arroyo near Valles"), all specimens were collected along the Pan American Highway between Tamazunchale, San Luis Potosi, Mexico, and a point approximately 44 km north of this town (figure 1). The samples come mainly from the Rio Moctezuma, from the Arroyo Palitla that flows into the Rio Moctezuma north of Tamazunchale, from the Arroyo Matlapa that enters the Rio Axtla from the south, and from the Rio Axtla proper or small streams running into it.

Fish of pedigree 1765 were collected in the Arroyo Palitla, 13 km north of Tamazunchale on April 21, 1965. Strain 38 has been derived from fish that were collected in the Rio Axtla, near the ferry crossing to Xilitla, in 1939. This stock has been maintained in the laboratory for 21 generations. An account of this stock has been presented by Kallman and Atz (1966). The system of raising fish and assigning pedigree numbers has been described previously (Gordon, 1950; Kallman, 1965). The four patterns with which this study is concerned are:

Cb: caudal blotch, a large oval area composed of micromelanophores in the proximal portion of the caudal fin (figure 2). The Cb pattern is also present in *X. m. montezumae* and *X. pygmaeus nigrensis* (Kallman and Atz, 1966).

Sc: spotted caudal, irregular longitudinal streaks or spots consisting of macromelanophores in the caudal fin (figures 3 and 4). This gene often gives rise to melanomas in a strain maintained in this laboratory (figure 5), but not in natural populations.

At: atromaculatus, a large number of black spots, composed of macromelanophores on flank and dorsal fin. Most spots concentrated below dorsal fin and in dorsal part of caudal peduncle (figures 3, 4, 5, 6).

Cam (the newly discovered pattern): carbomaculatus (from the Latin words *carbo* for coal and *maculatus* for spotted), relatively few but large spots on flank. This pattern differs from At in possessing fewer but larger markings. The dorsal fin is only rarely spotted and then only at the base (figures 2 and 7).

Individual spots were counted on all fish on the left side under an x10 dissecting scope. All fish were preserved in 10 percent formalin in the Genetics Laboratory for future reference. Size of fish is given in mm of standard length.

The distribution of the four patterns in natural populations was studied by examining the following preserved collections:

Arroyo Matlapa, San Luis Potosi, Mexico.

Gordon, Coronado, Gandy, April 14-15, 1939. UMMZ (University of Michigan, Museum of Zoology) #124374 (collected at Comoca).

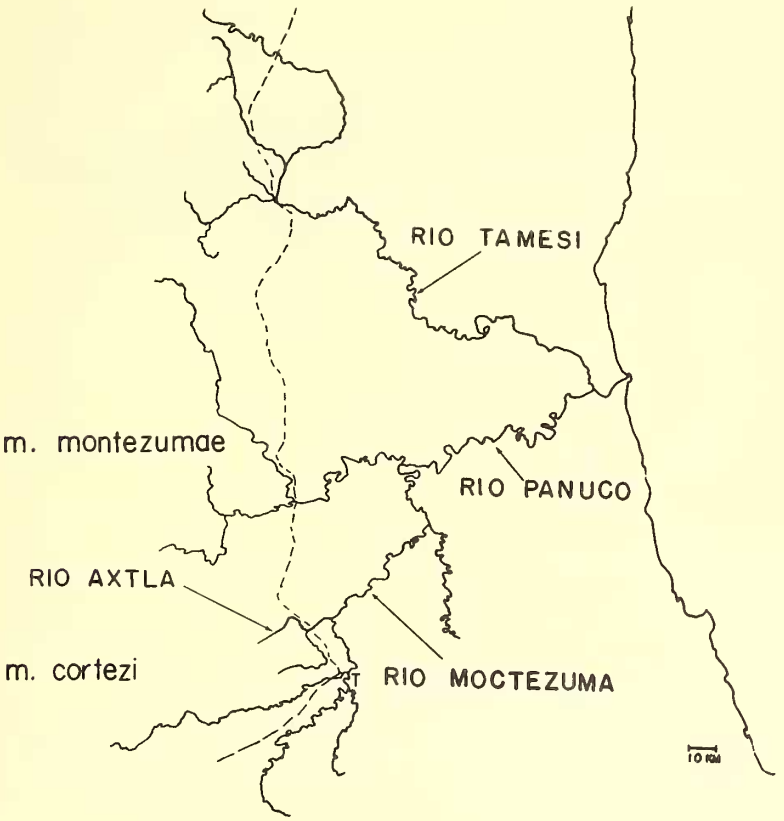

FIGURE 1. Rio Panuco-Tamesi system showing major streams. Virtually all collections of *X. montezumae cortezi* were made along a stretch of the Pan American Highway (broken line) between Rio Axtla and Tamazunchale (T).

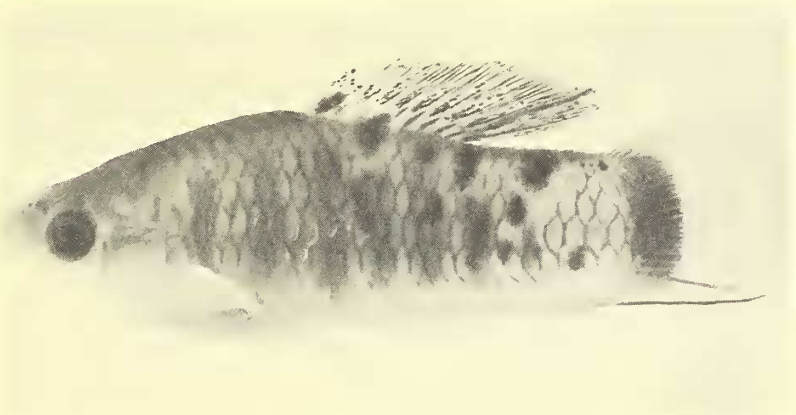

FIGURE 2. *X. montezumae cortezi*, ♂, 1765-11, 19 months after capture, 53 mm. The dark markings on the flank and the one large spot in the dorsal fin are caused by *Cam*. Note large size of the spots which often extend over several scale areas. The grayish elongate vertical bars ("parr marks") are under nervous control and are not part of Cam pattern. Such bars are found in most Montezuma swordtails. The dark crescent shaped area in the proximal part of the caudal fin is caudal blot, Cb.

FIGURE 3. *X. montezumae contezi*, ♂, strain 38, 20th laboratory generation, 12 months old, 35 mm. Heavy spotting on flank and in dorsal fin is At pattern. This pattern consists of relatively smaller spots than Cam. The irregular elongate streaks in caudal fin are caused by *Sc*, spotted-caudal.

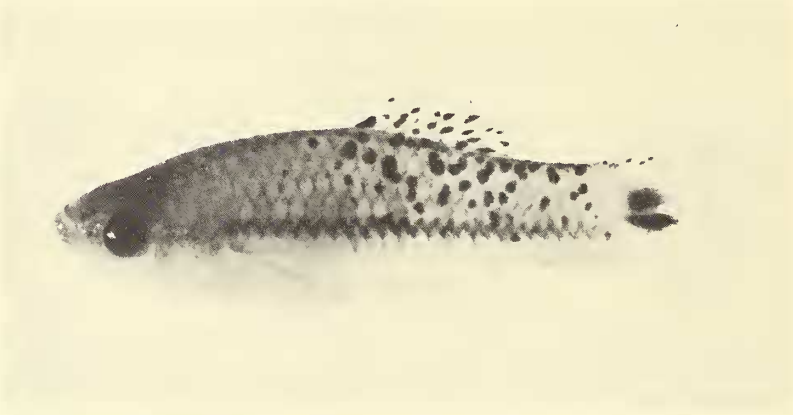

FIGURE 4. *X. montezumae cortezi*, ♀, strain 38, 19th laboratory generation, 19 months old, 44 mm. Spotting on flank and in dorsal fin is caused by At. Black mark in caudal fin is spotted caudal pattern.

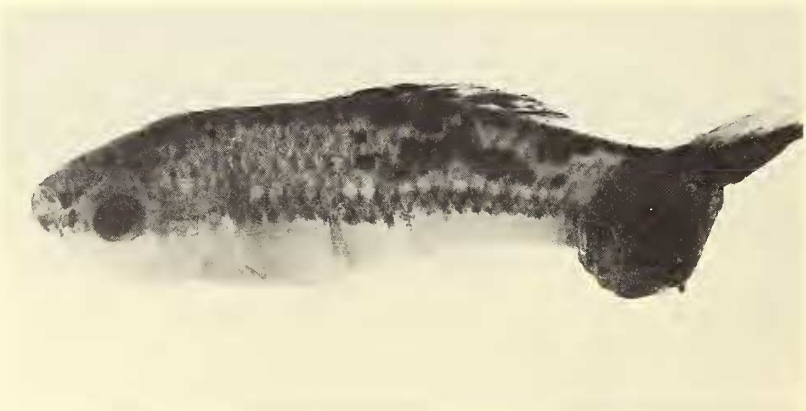

FIGURE 5. *X. montezumae cortezi*, ♀, strain 38, 21st laboratory generation, 19 months old, 42 mm. Spots on flank and in dorsal fin are caused by At. Large black area below anterior part of dorsal fin is the result of fusion of several smaller spots. Melanoma in caudal fin is caused by *Sc*.

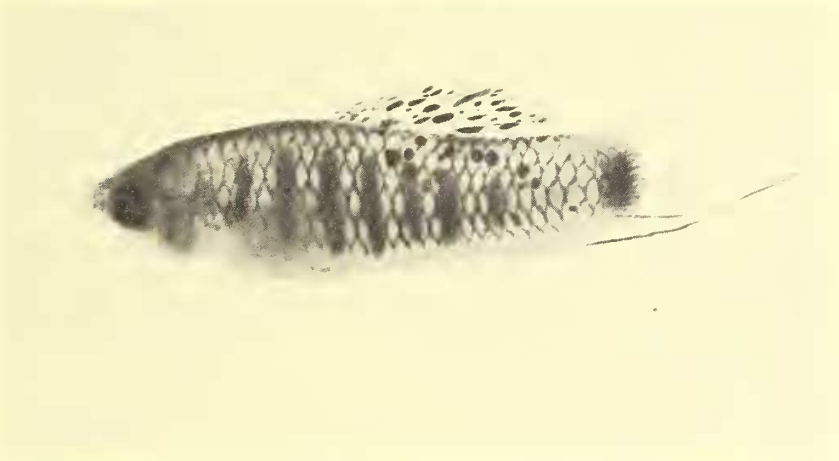

FIGURE 6. *X. montezumae cortezi*, ♂, ped. 2202, 12 months old, 34 mm. Markings on flank (17 spots) are attributed to *At* because of their small size. Caudal blot pattern is present in tail fin.

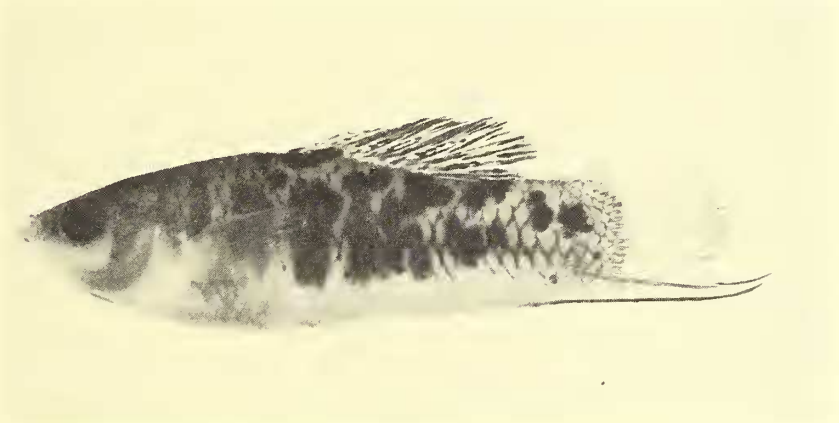

FIGURE 7. *X. montezumae cortezi*, ♂, ped. 2202, 12 months old, 43 mm. Spotting pattern on flank is attributed to *Cam*, since many of the spots (16) are of relatively large size.

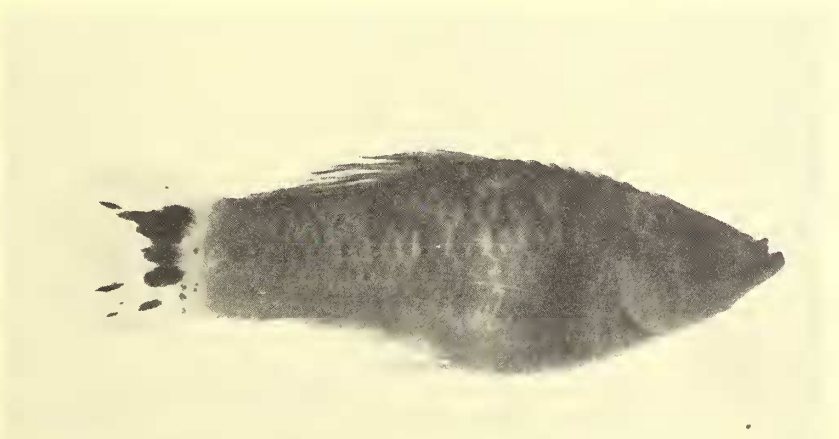

FIGURE 8. *X. variatus variatus*, ♂ UMMZ #108673, 33 mm. Black area in caudal fin is caused by macromelanophores and resembles spotted-caudal pattern of *X. montezumae cortezi*.

Gordon and party, April 14, 1939. UMMZ #124341 (collected at Matlapa).

Breder, Jr., March 25, 1940. UMMZ-Station 87 of New York Aquarium Expedition.

Rio Axtla and arroyo flowing into Rio Axtla, San Luis Potosi, Mexico.

Gordon, Whetzel, Ross, April 20, 1932. UMMZ #108602 (collected at Axtla).

Gordon and Atz, January 14, 1939. UMMZ #124174 (collected at Axtla).

Breder, Jr., March 25, 1940. UMMZ-Station 84 of New York Aquarium Expedition (collected in small arroyo between Rio Axtla and Rio Moctezuma).

Robinson, Nov. 26, 1957. UMMZ #174563 (collected 1 mile before Xilitla on road from Pan Am. Hwy.).

Arroyo Palitla, San Luis Potosi, Mexico.

Gordon and party, April 13, 1939. UMMZ #124331 (collected at Palitla).

Coronado, April 2, 1940. UMMZ #186323.

McLane, Dec. 19, 1940. UMMZ #162142.

Kallman and Kallman, April 21, 1965. Genetics Lab. collection.

Rio Moctezuma, at Tamazunchale, San Luis Potosi, Mexico.

Coronado, April 1, 1940. UMMZ #186319.

Sanders, July 11, 1937. UMMZ #180036, #105682.

Rio Calaboza (Rio Tempoal) system, Veracruz, Mexico.

Creaser, Gordon and Ostos, May 5, 1930. UMMZ #108678 (collected from Rio de los Hules, 11 miles SW of Tantoyuca).

Gordon, Creaser, Ostos, May 7, 1930. UMMZ #108679 (collected in tributary of Rio Calaboza, 20 miles S of Tantoyuca).

The frequencies of the patterns have been listed in Table 4, but only fish above 25 mm of standard length have been included. Smaller fish were deemed too young; in many the patterns were poorly developed and conceivably in others the pigment genes were not expressed. Three collections (numbers 105682, 108602, 124174) consisted exclusively of small specimens and none were included in the tables. No patterns were present in the four fish from the Rio Calaboza.

The age of preserved fish cannot be determined. Generally, size increases with age, but this does not hold true for males which virtually stop growing at the time of sexual maturity. Their sizes in the above collections ranged from 20 to 51 mm of standard length. It is a well known phenomenon in *Xiphophorus* that males (even siblings raised under identical conditions) become sexually mature at different ages (Rosen, 1960; Rosen and Kallman, 1969; Peters, 1964; Zander, 1965) and this accounts in part for the large variations in size.

Results

The results of all breeding experiments are listed in Table 1 and the postulated genotypes of the parental fish are given in Table 2.

The Cb pattern is inherited as a dominant autosomal trait. One male, 1765-13, collected from a natural population (ped. 1860) and two

Table 1.

Pedigree and phenotype of parents			
♀♀		♂♂	
1765-1	+	unknown	
1765-2	+	unknown	
1765-3	+	unknown	
38	At Sc	1765-13	Cb
38	At Sc	1765-11	Cam Cb
1797-1	+	1765-11	Cam Cb
1800-1	+	1800-12	Cb
1889b-1	Cb	1860-11	At Sc
1889b-2	Cam Cb	1962-11	+
1889b-4	+	1889a-11	At Sc
2085-1	Cb	1889a-11	At Sc
2043-1	Sc Cb	2085-12	Cam Cb
2096-1[4]	At	2085-11	Cb
2096-2[5]	Cam	2085-11	Cb
2043-2[6]	Cb	2085-13	Cam Cb
2043-3	+	2096-11	At
2096-3	+	2043-11	Sc Cb
2085-3	+	2085-14	+
2202-1	At	2214-12	Cb

[1] Age in months at which fish were scored for presence or absence of Sc.

[2] A strong Sc pattern obliterates Cb.

[3] 12 ♀♀ (2 Sc), 4 ♂♂ (3 Sc) at 8-10; 26 ♀♀ (4 Sc), 21 ♂♂ (3 Sc) at 12-15; 3 ♂♂ (1 Sc) at 19.

[4] Non-expression of *Sc* at 12 months.

[5] Non-expression of *Sc* at 17 months.

[6] Non-expression of *Sc* at 15 months.

fish bred in the laboratory, 2043-2 and 2043-11, the offspring of *Cb* parents, were homozygous for *Cb* (peds. 2258, 2277). All crosses of the type + x *Cb* yielded marked and unmarked progeny in equal frequency. When both parents were heterozygous, a ratio of 3 Cb : 1 + was obtained. A well developed Sc pattern that extends over much of the central portion of the caudal fin base can totally obscure or obliterate Cb, and this accounts for the absence of this pattern in some fish of peds. 1860 and 2277.

At is inherited as a dominant autosomal trait (peds. 2043, 2096, 2202, 2222, 2270, 2471) confirming the earlier report by Kallman and

INHERITANCE OF PIGMENT PATTERNS IN *Xiphophorus montezumae cortezi*.

Pedigree and sex of offspring		*Phenotypes of offspring*												*Age (month)*[1]
		At				Cam				+				
		+		Sc		+		Sc		+		Sc		
		+	Cb	+	Cb	+	Cb	+	Cb	+	Cb	+	Cb	
1797	♀♀	4								15				8 (incl. Sc ♂),
	♂♂	13								19		1		except 3 ♀♀, 19 ♂♂ at 12
1798	♀♀	1								5		1		8, except At ♀
	♂♂									5				at 12
1800	♀♀									5	5			5 ♀♀, 3 ♂♂ at 6,
	♂♂									9	17			5 ♀♀, 17 ♂♂ at 12, 6 ♂♂ at 16
1860	♀♀		27	2[2]	4									see[3]
	♂♂		21	1[2]	6									
1889a	♀♀	1	2											12
	♂♂	1	1	1										
1889b	♀♀						2			1	1			12
	♂♂						1							
1962	♀♀									3				9-12
	♂♂									3	2			
2043	♀♀	4	11	2	4					2	11	1	4	12-20
	♂♂	6	7	1	1					4	13		2	
2085	♀♀					1	3			2	6			9 except 4 Cb, 1 + ♀♀ and 1 + ♂ at 18
	♂♂					6	4			2	4			
2096	♀♀	4				1				2				8-12 except Cam
	♂♂	6				1		2		2				at 17
2202	♀♀	4	2	1	1	1	1	1	1	2	3	1	1	12
	♂♂	4	5			2	1		1	2	1			
2214	♀♀					2	6		3	3	8		2	13 except 2 Cb, 4 CbCam ♂♂ at 19
	♂♂					2	6				5			
2222	♀♀	7	6							4	6	2		10
	♂♂	5	6							5	11		1	
2249	♀♀					3	3	2		7	4	2		8
	♂♂					3	2		2	2	4	1		
2258	♀♀						9				7		2	16
	♂♂						4		1		12		1	
2270	♀♀	8		1						7		1		9
	♂♂	7								6				
2277	♀♀										10		2	18 except 7 fish
	♂♂										15	1[2]		(incl. 2 Sc) at 7
2319	♀♀									8				12-18 (3 ♀♀, 3 ♂♂),
	♂♂									7				21 (5 ♀♀, 4 ♂♂)
2471	♀♀	1	8	2			2	1		2	4			10
	♂♂		3			1	1				3			

Atz (1966). The results of ped. 2043 establish that the loci for *At* and *Cb* segregate independently. Under this assumption four classes of offspring should occur in the frequency of 3 *At Cb* : 3 *Cb* : 1 *At* : 1 + and the actual result fits this expectation rather well ($x^2=3.11$; n=3; $0.5>p>0.3$). The alternate possibility that the two loci are linked is ruled out by the presence of wild-type progeny.

The Cam pattern is inherited as a dominant trait that is not associated with sex. There exists no statistically significant difference in the frequency of Cam males and females regardless from which parent the pigment factor was introduced (Cam from P_1 ♀: Cam — 12 ♀♀, 16 ♂♂; + — 21 ♀♀, 14 ♂♂, combined count of peds. 2085, 2249; Cam from P_1 ♂: Cam — 22 ♀♀, 14 ♂♂; + — 24 ♀♀, 20 ♂♂, combined count of peds. 1889 b, 2214, 2258). Crosses of the type *Cam Cb* x ++ give rise to four classes of offspring indicating that the two pigment genes are not linked (ped. 1889b, 2085).

The spotted patterns, At and Cam, are controlled by two factors occupying different loci that are not linked. Crosses of an unspotted parent with one that had inherited both factors yielded offspring in the frequency of 2 At : 1 Cam : 1 + (41 At, 17 Cam, 23 +, combined count of peds. 2096, 2202, 2471). By contrast, all crosses of the type spotted x unspotted which gave rise to either At or Cam progeny, but not both, yielded marked or unmarked fish in a ratio of 1 : 1 (65 Cam, 77+, combined count of peds. 1889b, 2085, 2214, 2249, 2258; 76 At, 79+, combined count of ped. 2043, 2222, 2270). It must be pointed out that not one At progeny was obtained from two of the crosses (Cam x + , peds. 2214, 2258) in which the wild-type parent had come from pedigrees with At offspring and, conversely no Cam fish were present in ped. 2222, although some sibs of the + parent were Cam. The results of these crosses do not support the hypothesis that the difference between the two spotted patterns are caused by modifiers.

Fish that are genotypically *At Cam* look like fish with just *At*. Two attempts were made to determine whether the more heavily pigmented fish contained both factors while the more lightly pigmented ones were merely *At*. Male 1889 a-11 was thought to possess both factors and this proved to be the case. However, female 2202-1, which looked just like any other At fish of pedigrees in which Cam did not occur, was heterozygous for both factors. Of critical importance for establishing that *At* and *Cam* are not allelic was the demonstration that a wild-type female of ped. 2096 did not carry a spotted factor unexpressed (ped. 2277). Thus the wild-type fish of ped. 2096 cannot be attributed to nonexpression of a pigment gene.

The inheritance of *Sc* is difficult to study because of its incomplete penetrance. From an inspection of Table 1 (and also of Tables III and IV of Kallman and Atz, 1966) it may appear that Sc has a polygenic basis. In a sense this is true, since obviously many modifiers are involved in bringing about the pattern. However, the series of crosses in which *Sc* was introduced into a *X. hellerii* genotype (Kallman and

Table 2. Genotypes of Parents from Matings of Table 1

Pedigree	♀♀								♂♂							
1797	+	+	+	+	+	+	+	+	unknown							
1798*	+	+	(*Sc?*)	+	+	+	+	+	unknown							
1800	+	+	+	+	+	+	+	+	unknown							
1860	*At*	*At*	*Sc*	*Sc*	+	+	+	+	+	+	+	+	+	+	*Cb*	*Cb*
1889a	*At*	*At*	*Sc*	*Sc*	+	+	+	+	+	+	+	+	*Cam*	+	*Cb*	+
1889b	+	+	+	+	+	+	+	+	+	+	+	+	*Cam*	+	*Cb*	+
1962	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Cb*	+
2043	+	+	+	+	+	+	*Cb*	+	*At*	+	*Sc*	+	+	+	*Cb*	+
2085	+	+	+	+	*Cam*	+	*Cb*	+	+	+	+	+	+	+	+	+
2096	+	+	+	+	+	+	+	+	*At*	+	*Sc*	+	*Cam*	+	+	+
2202	+	+	+	+	+	+	*Cb*	+	*At*	+	*Sc*	+	*Cam*	+	+	+
2214	+	+	*Sc*	+	+	+	*Cb*	+	+	+	+	+	*Cam*	+	*Cb*	+
2222	*At*	+	*Sc*	+	+	+	+	+	+	+	+	+	+	+	*Cb*	+
2249	+	+	*Sc*	+	*Cam*	+	+	+	+	+	+	+	+	+	*Cb*	+
2258	+	+	*Sc*	+	+	+	*Cb*	*Cb*	+	+	+	+	*Cam*	+	*Cb*	+
2270*	+	+	(*Sc?*)	+	+	+	+	+	*At*	+	(*Sc?*)	+	+	+	+	+
2277*	+	+	(*Sc?*)	+	+	+	+	+	+	+	*Sc*	+	+	+	*Cb*	*Cb*
2319	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2471*	*At*	+	(*Sc?*)	+	*Cam*	+	+	+	+	+	(*Sc?*)	+	+	+	*Cb*	+

* In these pedigrees one or both parents must have been heterozygoys for *Sc*.

Atz, 1966) clearly shows that this pattern is due to a single major pigment gene. *Sc* does not appear to have been present in five related pedigrees (peds. 1800, 1889b, 1962, 2085, 2319), since none of the 94 fish exhibited the pattern.

Strain 38 is apparently homozygous for *Sc*. The pattern is present in about 77 percent of the fish (10th to 15th generation, Kallman and Atz, 1966; 16th to 21st generation, Table 3) and those that do not develop it nevertheless carry the *Sc* gene as shown by breeding experiments (Kallman and Atz, 1966). This was also the case with the female parents of peds. 2222, 2249, and 2258, and with one or both parents of peds. 2270 and 2471 (Table 1). The expression of Sc in strain 38 varies from a small elongate spot in the caudal fin to large melanomas that eventually destroy the fin (Atz, Kallman, and Nigrelli, 1963) (figure 5). This stock represents the only known example in *Xiphophorus* in which melanomas caused by macromelanophore genes occur without prior hybridization or cannot be attributed to mutation or crossing over (Kallman and Schreibman, 1971). There is some evidence that both the incidence and also the degree of Sc expression increases with age. The highest percentage of Sc individuals and tumorous fish observed was in the three sibships of the 21st generation which were maintained for longer periods of time than any other generation (Table 3). Four males did not develop the Sc pattern until they were older than 17 months. However, Sc melanomas are not only found in older fish; large tumors may be present in fish as young as seven months. The Sc melanoma invariably arises in the proximal portion of the tail fin, but may eventually encompass the entire fin and also part of the caudal peduncle. In the most severe cases, the caudal fin sloughs off. Fish with a large melanoma have a high mortality and often die several months or years sooner than their sibs without tumors. The first extant record of a Sc melanoma comes from the seventh laboratory generation. When fish of strain 38 are outcrossed to other stocks of *X. m. cortezi,* the penetrance of *Sc* becomes reduced to approximately 22 to 30 percent. This estimate is based upon two pedigrees (1860, 1889a) in which all individuals were heterozygous for *Sc* and seven others (2043, 2096, 2202, 2214, 2222, 2249, 2258) in which one-half of the fish were expected to have inherited *Sc* (Table 1).

No association of *Sc* with sex is apparent. Assuming an XX ♀♀ - XY ♂♂ mechanism for *cortezi,* fish of peds. 1860 and 1889a must have inherited Sc on the X chromosome. Conse-

Table 3. Pigment Patterns in *Xiphophorus montezumae cortezi,* Strain 38 (16th to 21st Generations; Obtained from Matings of At Sc ♀♀ x At Sc ♂♂)

Generation	At		At Sc		*Total*		*Age*[1]	*Tumors*[2]
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂		
16A	5[3]	2	11	13	16	15	12	1
16B		1	5	4	5	5	12	0
16C	1	1	1	3	2	4	10	0
17A	2	5	5	9	7	14	9	1
17B	1		4	5	5	5	10	0
17C	3		7	5	10	5	9-12	1
18A	3	2	4	6	7	8	9-12	3
18B	2	1	3	5	5	6	11	2
18C	1	2	5	4	6	6	11, 17[4]	0
18D	1	–	4	4	5	4	11	2
19A	1	2	1	2	2	4	7	1
19B	2	–	8	7	10	7	12	2
19C	4	3	3	7	7	10	11	1
20A	–	2	2	2	2	4	8	2
20B	3	3	1	2	4	5	8	1
20C	2	1	10	12	12	13	12	5
21A	–	–	6	8	6	8	16	3
21B	3	–	14	11	17	11	17[5]	5
21C	1	–	15	11	16	11	16	15
Total[6]	35	25	109	120	144	145		

[1] Age in months at which fish were scored for presence or absence of Sc.

[2] This column does not include fish that were merely melanotic.

[3] Three of these fish may have had Sc, but spots were of small size and could conceivably be part of At.

[4] ♀ at 17 month, ♂♂ at 11 months.

[5] Four males showed no Sc at 17 months, but when rescored a year later the pattern was present in all of them.

[6] For earlier generations, see Kallman and Atz, 1966.

quently, in peds. 2043, 2096, and 2202, *Sc* should have been inherited in females only. However, the spotted-caudal pattern was present in both sexes. The difference in the percentage of Sc males and females is not statistically significant (because of the small number of Sc fish, the results of all three pedigrees have been combined: ♀♀: 16 Sc of 70, 22.9 percent; ♂♂: 7 Sc of 61, 11.5 percent, P=0.09).

Similarly no sex linkage of Sc can be demonstrated, if the sex determining mechanism of *cortezi* is assumed to be of the WY ♀♀ - YY ♂♂ type. Under this condition Sc males and females are expected in peds. 2043, 2096, and 2202, but no Sc females should occur in peds. 2214, 2222, 2249, and 2258. This was not the case. If the data of all four pedigrees are combined, no significant difference between the frequency of Sc males and females is observed (♀♀: 13 Sc of 88, 14.8 percent; ♂♂: 6 Sc of 73, 8.2 percent; P = 0.19).

Sc and *Cam* are not linked. The Cam pattern of ped. 2096 can be traced to 1765-11 and Sc to strain 38. It the patterns were controlled by factors on homologous chromosomes, no *Sc Cam* progeny should be present in ped. 2249. For similar reasons, the *Sc* and *At* loci cannot be linked (already reported by Kallman and Atz, 1966), since in peds. 2043 and 2202, some fish inherited both.

According to the results of ped. 2277, male 2043-11 was homozygous for *Cb* and heterozygous for *Sc*. Since 2043-11 inherited one of its *Cb* factors from 1765-11 and the other from 1765-13 while *Sc* can be traced to strain 38, the loci for *Sc* and Cb cannot be located on homologous chromosomes.

At and Cam patterns can be distinguished in sexually mature fish on the basis of number and size of spots. Cam fish have few large spots on the flank (from 1 to 16 in our experiments), and at most one or two large spots in the proximal portion of the dorsal fin. More than half of the markings of the Cam pattern, even in young individuals, are at least as large as one hexagonal unit that marks the reticulum. Often the spots extend over several scale areas. However, there is a brief period when Cam is just developing during which the size of the Cam spots is identical with those of At. By contrast, the At pattern consists of from one to several dozen small spots on the flank and in the dorsal fin. In At fish that are 12 months or older, the number of spots may become so large that adjacent spots fuse to form large irregular black patches below the dorsal fin. Such markings may become as large as those of Cam fish. This is one of the reasons why *At Cam* individuals look like those with just *At*. All pedigrees listed in Table 1 and figure 9 were scored independently for both patterns by the author and one or two laboratory assistants with identical results in all cases. Even among offspring of crosses of the type *At Cam* x ++ (identified by a ratio 3 spotted:1 unspotted) *Cam* fish could be separated from *At* or *At Cam* progeny (peds. 2096, 2202, 2471). For example, the male of ped. 2202 with 16 spots was classified as *Cam* on the basis of large spot size and absence of spotting from the dorsal fin, while the two males with 17 spots were *At* because the size of their spots was small (figures 6 and 7). For the same reason the female of ped. 2471 with ten spots was classified as *Cam*. It must also be pointed out that the size of the spots of all fish listed as Cam in Table 1 and figure 9 was similar to that of the fish illustrated in figures 2 and 7. Observations on the etiology of the Cam pattern in ped. 2258 has shown that the small number of large spots is not due to a fusion of several smaller spots. Although when different pedigrees are compared with each other, the spot number (but never their size) of the more heavily pigmented Cam fish may overlap with the least pigmented At fish, no such overlap was observed in the three crosses in which both *At* and *Cam* segregated. It is significant that in ped. 2096 the Cam progeny had fewer spots than the At fish, although the former were scored at 17 and the latter at 12 months.

The number of spots that compose the At pattern increases with age. This is clearly illustrated by fish of ped. 1860 scored at different ages and also by a comparison of 9 to 10 months old fish of peds. 2222 and 2270 with 12 to 20 months old ones (ped. 2043). No such increase has been noted in *Cam* fish (compare peds. 2085, 2249, 8 to 9 months, with peds. 2214, 2258, 13 to 19 months).

X. m. cortezi with macromelanophore patterns have been repeatedly illustrated in the past but no distinction between At and Cam has ever been made. Only Zander (1969) has suggested that two slightly different spotting patterns may occur in *X. m. cortezi*. Photographs of fish with typical *At* can be found in Gordon (1951, Fig. 17, ♂; 1956, lower cover picture; 1957, Fig. 13, ♀ and ♂), Kosswig (1936, Abb. 3, ♀), Stöwahse and Villwock (1969, Fig. 1a, ♀), and Zander (1967, Tafel V, Abb. 18, ♀). Fish with typical *Cam* have been illustrated only by Rosen (1960, Fig. 13, ♂) and Zander (1967, Tafel V, Abb. 18, ♂). The picture of *X. m. cortezi* on page 10 of Gordon (1956) presumably refers to At as judged by the small size of the spots, but their low number and their absence from the dorsal fin makes it a somewhat doubtful identification. The phenotype of the male illustrated by Kosswig (1935, Abb. 1) is also doubtful because of the presence of only a single large spot.

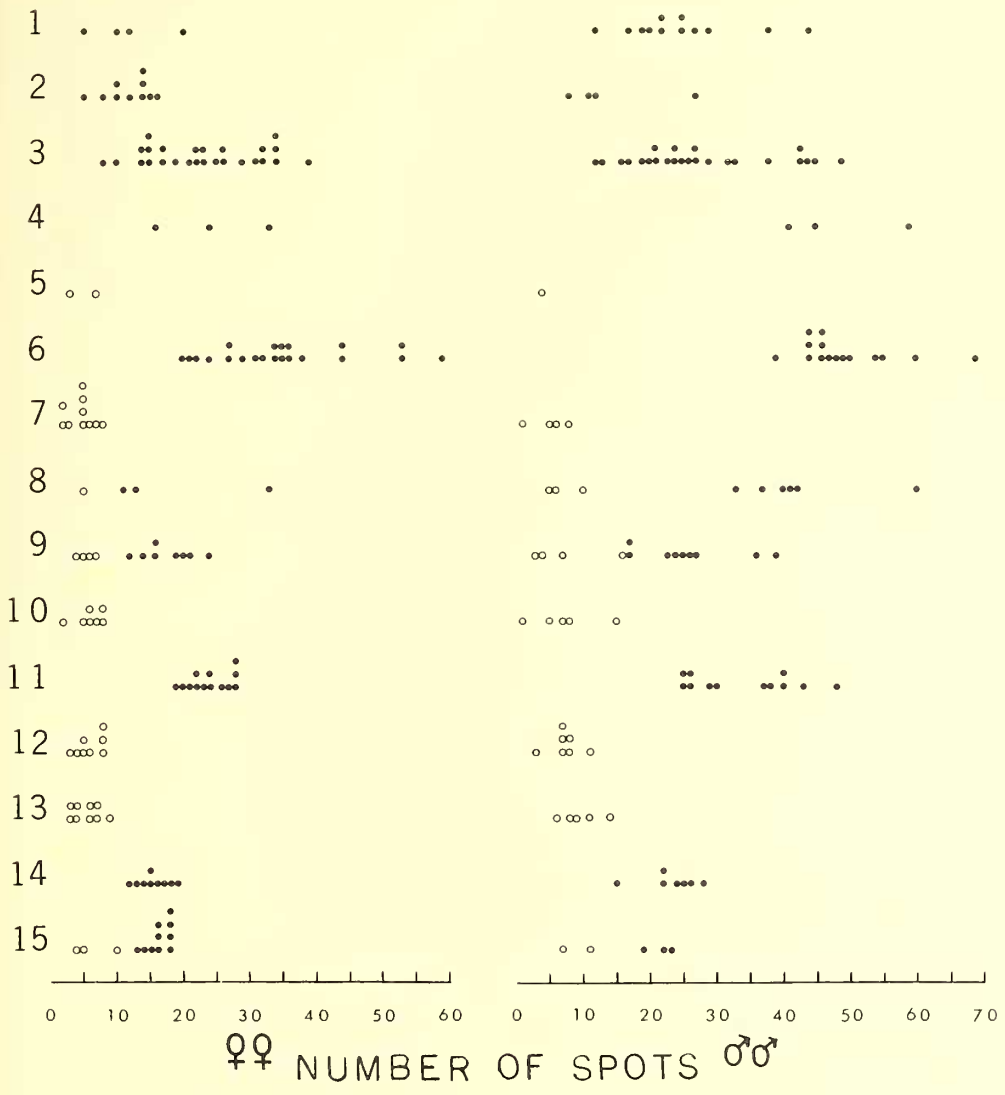

FIGURE 9. Number of spots comprising *At* and *Cam* patterns in *Xiphophorus montezumae cortezi* (laboratory broods). The ages at which the fish were scored are given in parenthesis (months). The number of spotted offspring of certain pedigrees is sometimes less than in Table 1, because some fish were used in other experiments or died (not preserved) before the spots were counted.

1) ped. 1797 (8-12)
2) ped. 1860, ♀♀ (8-10), ♂♂ (10)
3) ped. 1860, ♀♀ (12-15), ♂♂ (12-19)
4) ped. 1889a (12)
5) ped. 1889b (12)
6) ped. 2043 (12-20)
7) ped. 2085 (9)
8) ped. 2096, *At* ♀♀ (8-12), *At* ♂♂ (12), Cam ♀♀, ♂♂ (17)
9) ped. 2202 (12)
10) ped. 2214, ♀♀ (13), ♂♂ (19)
11) ped. 2222 (10)
12) ped. 2249 (8)
13) ped. 2258 (16)
14) ped. 2270 (9)
15) ped. 2471 (10)

In preserved collections from natural populations, two types of spotted patterns can be distinguished that correspond to Cam and At of laboratory reared fish (Table 3). Both patterns are present in the Rio Moctezuma, Arroyo Palitla, Arroyo Matlapa, and Rio Axtla (except UMMZ 174563), but their frequencies are not the same in the different collections. Whether these are true genetic differences between adjacent local populations or are merely due to sampling error cannot be determined. Cam fish were absent from one arroyo flowing into the Rio Axtla but made up 28 percent of the fish from the Arroyo Matlapa. The frequency of At fish in the individual samples ranged from 11 to 40 percent.

Between 25 and 32 mm of standard length, the number of spots of Cam and At patterns overlaps considerably (figure 10), but even within this range, Cam fish had usually fewer markings than those with At. Fish listed as Cam and those recorded as At with 10 or more spots have probably been correctly identified, but some of the individuals scored as At with fewer than 10 spots could conceivably have been Cam in which the pattern was just beginning to develop. Below 25 mm of standard length, only few fish can be classified unequivocally as to their pattern and, therefore, they have been omitted from Table 3 and figure 10. In larger (presumably older fish) the number of spots of At and Cam fish diverges strongly (figure 10). These data are in agreement with laboratory observations that with age the number of markings increases in At fish only.

Sc and Cb are also found in the four main locations, but Cb was absent from two collections. Cb appears to be most common in the Arroyo Palitla. The frequency of fish with Sc ranged from 6 to 43 percent.

Discussion

The genetic analysis of the spotted phenotypes of *X. m. cortezi* indicates that this form has three unlinked macromelanophore loci, each possessing the wild-type (unmarked) and one pattern allele. To this author, it seems unlikely that additional patterns will be discovered in the area of the Rio Moctezuma, Rio Axtla, and Arroyo Palitla, because in the extensive collections during the last 40 years only Cam, At, and Sc were present. The four patterns occur in all of the populations sampled. Nothing is known about the populations (if any) that live upstream from Tamazunchale in the Rios Moctezuma, Amayac, and Clara, or in the Rio Calaboza system. The evolutionary events are unknown that are responsible for the difference between *maculatus, variatus,* and *milleri* in which the numerous macromelanophore factors are all members of the same locus or supergene, and *X. m. cortezi.* Kallman and Atz (1966) have pointed out that no experiment exists that would indicate whether *At* or *Sc* or both are homologous to the macromelanophore factors of the above three species or are of independent

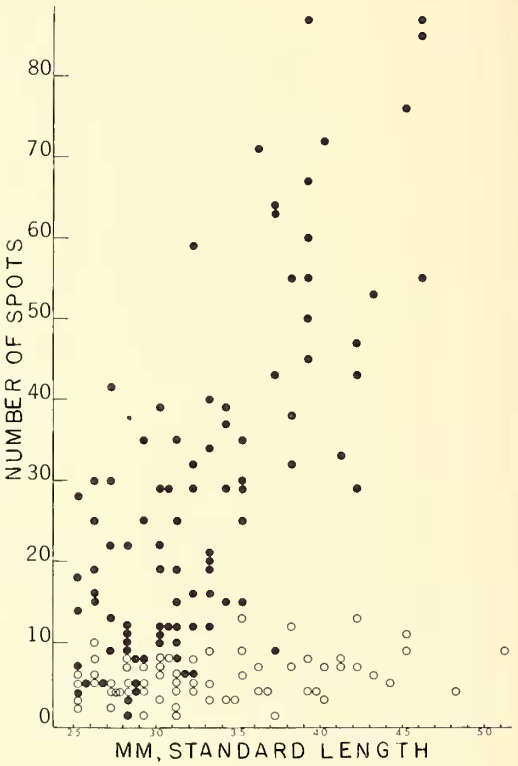

Figure 10. Number of spots comprising At (solid) and Cam (circle) patterns in *X. montezumae cortezi* from natural populations. There is considerable overlap in smaller fish, but above 30 mm of standard length Cam fish have fewer spots than fish with At. Larger fish with At have more spots than smaller individuals. The number of Cam spots is independent of size.

origin. To these considerations we may now add *Cam.* An alternate possibility mentioned by Kallman and Atz (1966) and Zander (1969) is that the macromelanophore factors of *cortezi* are genetically related to those of *maculatus* and were perhaps at one time members of the same complex macromelanophore locus. However, during the course of evolution they could have become separated through chromosomal rearrangement and are now located on different chromosomes. Support for such a view is provided by the observations of Kallman and Schreibman (1971) and MacIntyre (1961) that in *maculatus* the macromelanophore factors can become separated through crossing-over. However, admittedly such crossover events within the locus are rare.

For an understanding of the evolution of the macromelanophore systems, it is also important to determine whether any of the patterns of *X. m. montezumae* are controlled by genes that are identical with *At, Cam,* or *Sc.* Preliminary results obtained in this laboratory indicate that at least some patterns of *X. m. montezumae* are sex-linked. Zander (1969) has reported that in a complicated hybrid involving *maculatus, hellerii,* and *montezumae,* a pattern of *X. m. montezumae* that he called *Sr,* was located on a chromosome that segregated from the sex chromosome of *maculatus.* If Zander's (1969) experiment can be confirmed, then four species of *Xiphophorus* are known with macromelanophore loci located on the same pair of homologous chromosomes. Since the diploid number of *Xiphophorus* is 24 (Friedman and Gordon, 1934; Lueken and Foerster, 1969), Zander's result can be taken as additional evidence for the suggestion that the macromelanophore genes of the various species can be traced to a common ancestral form.

Of particular interest is the relative high frequency of *Sc* in natural populations. This gene more than any other has to be considered as potentially deleterious, since within strain 38 it may give rise to melanomas. This strain of *X. montezumae cortezi* represents the only known example in the genus in which atypical pigment cell growths caused by a macromelanophore gene, *Sc,* occurs without prior hybridization, or cannot be attributed to mutation or crossing-over. If the penetrance of *Sc* is as low in natural populations as in the laboratory pedigrees of Table 1, the incidence of *Sc* must be considerably higher in natural populations than appears from Table 4.

Assuming a penetrance of 30 percent and no difference between homozygous and heterozygous individuals, about 83 percent of the fish from the Rio Axtla must carry *Sc.* Based upon this rather rough estimate, the frequency of this potentially injurious gene may be as high as 59 percent. Presumably a large number of modifiers are present in natural populations that keep the expression of *Sc* under control. No *X. m. cortezi* with melanotic fins or melanomas have been seen in preserved collections. This by itself cannot be taken as proof that fish with genotypes permitting the development of tumors do not occur in nature. Undoubtedly, such fish will be swiftly eliminated by predators as the melanoma develops and their swimming ability becomes impaired, and thus there will be no record. More convincing evidence for the absence or extreme rarity of such gene combinations comes from the analyses in the laboratory of broods from wild-caught females or from hybrids between strain 38 and wild stock. No melanomas were seen in such offspring (the data from Table 1 of this investigation and from Table 3 of Kallman and Atz, 1966). The presence of tumors in strain 38 must be a product of selection in the laboratory. Although no deliberate attempt has ever been made to increase the incidence of melanomas, it has consistently been the practice of our laboratory assistants to choose as parents for the following generation fish with well developed Sc patterns at the age of 9 to 10 months.

The only other pattern similar to Sc of *X. montezumae cortezi* is known from the populations of *X. variatus variatus* inhabiting the Rio Cazones system (Rosen, 1960). Morphologically this pattern (figure 8) is indistinguishable from that of *cortezi.* Based upon two collections (Gordon, Atz, Whetzel, station 45, March 31, 1948; Arroyo Mariandrea at Mariandrea, Puebla, Mexico, about 10 miles W of Poza Rica on road to Apapantilla; Gordon, Creaser, Ostos, UMMZ #108673, May 11, 1930, unnamed arroyo, near Agua Fria, 12 miles S of Miahuapan), this pattern is present in approximately 10 percent of the population. No Sc-like pattern was seen in any other *X. variatus* collection (for complete list, see Rosen, 1960). According to Atz (1962) and Zander (1969), the expression of *Sc* of *X. m. cortezi* is suppressed when introduced into *X. v. variatus* [*variatus* from Rio Axtla (Atz) and of unknown geographic origin (Zander)]. The spotted-caudal patterns of *variatus* and *cortezi,* therefore, may be caused by different genes. The crucial test, however, can only be provided by introducing the factors responsible for the patterns in the two species on to common genetic backgrounds.

Closely related to the problem of the polymorphic macromelanophore (and xanthoerythrophore) patterns is the one concerned with the evolution of mechanisms for sex determination. As was pointed out in the introduction, the gonosomes of three species, *X. variatus, X. milleri,* and *X. maculatus,* are

homologous. The first two have a XX ♀♀ — XY ♂♂ mechanism, while in natural populations of *X. maculatus,* three types of females, XX, WX, WY, and two types of males, XY and YY, may occur (Kallman, 1965 and 1970a). Although crossing over between the Y and W chromosomes has been observed repeatedly in the laboratory, no marked W chromosomes have yet been found in wild populations (Kallman, 1970a and unpublished). Macromelanophore and other pigment factors, however, are present on the X. Zander (1968) has recently discovered two pigment factors, *Vfl* and *Fl,* in two populations of *X. pygmaeus nigrensis* that have only minimal phenotypic effects within their own species, but manifest themselves as strikingly

TABLE 4. MELANOPHORE PATTERNS IN WILD POPULATIONS OF *Xiphophorus montezumae cortezi*
Patterns in preserved fish

Location		+	Cam	At	Sc	Cb	At Sc	Cam Sc	Cb Sc	Cb At	Cam Cb	Cam Sc	Cb At Sc	%
Axtla														
Sta. 84 N.Y.A.	♀♀	19	3	16		2				1				Cam 5
	♂♂	7	3	6	5		2	1		2				At 36
174563	♀♀	9		8	14		4							Sc 26
	♂♂	13		8	6		2							Cb 4
Matlapa														
124374	♀♀	14	13	7		2	1				2			Cam 28
	♂♂	23	9	2	6	3					1	1		At 17
Sta. 87 N.Y.A.	♀♀	8	3		1	2								Sc 14
	♂♂	8	6	3	1	1		3		1				Cb 8
124341	♀♀	7	3	6	2									
	♂♂	8	2	4	3		3	1						
Palitla														
124331*	♀♀													
	♂♂	4		6	3	1	1	2		2				Cam 14
16242	♀♀	13	2	5	3	4	3			1	1			At 29
	♂♂	11	2	11	9	2	2	1		3	1	1	1	Sc 23
186323	♀♀	1												Cb 20
	♂♂	8	3	2	1	1	2		2		1			
K & K, 1965	♀♀	5		2		1		1		1				
	♂♂	4	1			1					2			
Moctezuma														
180036	♀♀			1										Cam 20
	♂♂		1	1										At 33
186319	♀♀	4	1	1										Sc 20
	♂♂		1	1	2	1	1							Cb 7

*Not included in percentage calculations, since this is obviously a selected sample. This collection consisted of 319 specimens, but only 19 mature males are extant.

red body patterns after introgression into *X. maculatus*. Most significant is that *Vfl* is located on a chromosome that is homologous to the Y chromosome of *maculatus* and can replace it functionally. Although no numbers were reported, Zander's experiment suggests that in one of his *nigrensis* stock, sex-determination is by the XX ♀♀—XY ♂♂ mechanism, since all males but none of the females inherited *Fl*. Because of the homology of the sex chromosomes of *maculatus, variatus,* and *milleri,* it is unlikely that the same pair of undifferentiated chromosomes had evolved independently into gonosomes three times (or four times if *X. pygmaeus nigrensis* is included). Presumably, the ancestral species already had a XX-XY mechanism with a macromelanophore locus. The W chromosome of *maculatus* may be a recent innovation (Kallman, 1970a).

The sex determining system of *X. hellerii* is not well understood because of widely fluctuating sex ratios (Kallman and Atz, 1966; Peters, 1964). Several authors are of the opinion that sex determination in this genus has evolved from an ancestral condition in which sex determination was achieved polygenically by the segregation of many M or F factors scattered over many chromosomes to one with well defined gonosomes (Anders and Anders, 1963; Dzwillo and Zander, 1967; Gordon, 1952; Kosswig, 1964; Peters, 1964). According to these investigators, the original condition, or one similar to it, is still present today in *X. hellerii*. An alternate possibility has been suggested by Kallman (1965, 1968) and Kallman and Atz (1966).

It is therefore of considerable interest that an early experiment (but unfortunately based upon hybrids of unknown origin with the identity of some of the relevant pigment genes in doubt) by Breider and Mombour (1949) suggested that the chromosome of *cortezi* that carries *Sc* may be homologous to the sex chromosomes of *maculatus*. The crosses listed in Table 1, limited as they are because of the low penetrance of *Sc*, do not indicate sex linkage for *Sc*. This does not preclude the possibility, however, that the chromosome on which *Sc* is located is homologous to the gonosomes of *maculatus* and the other species, but that within *X. m. cortezi* this chromosome plays no role in sex determination.

The data published previously by Kallman and Atz (1966) did not provide any evidence for or against the presence of a XX ♀♀—XY ♂♂ or any other sex chromosome mechanism in *X. m. cortezi*. Of the forty pedigrees reared in the Genetics Laboratory only two showed a significant deviation from unity. Similarly, Kosswig (1959) did not find any significant preponderance of one or the other sex. Only Zander (1965) reported some crosses in which virtually all offspring differentiated into females (e.g. one male mated to three females sired 105 offspring, all but two females; a second male mated to the same three females gave rise to 183 females and one male). According to his interpretation, *X. m. cortezi* possesses an XX ♀♀—XY ♂♂ sex determining mechanism that can easily be upset by autosomal factors. Males that sire predominantly female broods have two X chromosomes and a certain number of autosomal male determining factors that override the action of the sex chromosomes. The male determining potency of the Y chromosome of *cortezi* is less than that of the Y of *maculatus* (Zander, 1965).

Zander pointed out that *X. m. cortezi* may have reached a stage in the evolution of sex-determining mechanisms that is intermediate betwen a polygenic (original) one and one in which sex is determined strictly by gonosomes. In the *Sc* males of his experiments, sex determination was thought to have been by sex chromosomes (XY ♂♂), but in the males without *Sc* to have proceeded polygenically (XX). However, the significance of these relationships was not clear (Zander, 1965).

Females with the exceptional sex genotype XY and males that are XX are known from *X. maculatus* and other species (see summary by Kallman, 1968), and there is no reason to assume that this condition cannot occur in *cortezi* as well, if indeed it has a XX-XY system. The abnormal sex ratio of 288 ♀♀ : 3 ♂♂ is certainly strong evidence that the male parents of these broods possessed a sex genotype more characteristic for females, but, curiously, when these males were mated to other females normal sex ratios were obtained.

Because the sex chromosomes of *X. maculatus* carry dominant sex-linked marker genes, the recognition of genetic sex reversals (i.e. XX ♂♂ and XY ♀♀) presents no problem in this species. This is not the case for *X. m. cortezi* and many other species. In this context it must be pointed out that significant deviations from an expected 1:1 sex ratio cannot *a priori* be attributed to genetic sex reversals as has been done by Schröder for *Poecilia* (1964) and Zander for *X. m. cortezi*. Other independently arrived corroborative evidence, e.g. sex-linked marker genes or chromosome analysis, is needed in each case. Kallman (1965) working with *X. maculatus* found that the deviation from the expected sex ratio (ped. 1485, 23 XX ♀♀, 44 XY ♂♂, Table 15) was due to the deficiency of one class and not due to genetic sex reversals.

To me the conclusion is not justified that XX males are not exceptional for *cortezi* and occur rather frequently (XX-Ausnahme-♂♂ sind für *cortezi* keine Besonderheit und treten relativ häufig auf), since nowhere did Zander (1965)

indicate just how frequently broods with a significant excess of females are obtained. Nothing was reported about the sex ratios or breeding performance of his stock. The data of Kallman and Atz (1966) and Kosswig (1959) do not show that unbalanced sex ratios are of common occurrence in *cortezi*.

Zander's interpretation was in part based upon the analysis of sex ratios of hybrids between *cortezi* and *maculatus* and *cortezi* and *variatus*. Sex determination in species hybrids of *Xiphophorus* while sometimes proceeding normally, as e.g. in *maculatus* x *pygmaeus* hybrids (Zander, 1968), is just as often contrary as to what one would expect from the sex chromosome genotype of the hybrids (provided one uses species with marked gonosomes which is not the case with *cortezi*). Kallman and Atz (1966) found that in *maculatus* x *milleri* hybrids, sex determination was largely governed by the sex chromosomes (XX ♀♀ XY ♂♂) provided the *maculatus* parent came from the Gp stock. But when the *maculatus* parent came from stock Hp-2, more than half of the XX offspring differentiated into functional males, which upon breeding with XX females of either species yielded progeny (large numbers) with a sex ratio that approached unity, mimicking XX ♀♀ x XY ♂♂ crosses. Other examples of atypical cases of sex determination are provided by Zander's (1968) *milleri-pygmaeus* crosses and by the *hellerii* x *maculatus* hybrids (summary in Table 26, Kallman, 1965). Even Zander (1965) admits that some of the results of his hybrid crosses fit into his scheme of sex determination for *cortezi* only with difficulty or not at all.

The crosses listed in this paper do not help to clarify the sex chromosome mechanism of *X. m. cortezi*, because no sex-linked marker genes have been found. However, these results, as our earlier ones, clearly show that males (XX ?) that sire predominantly female broods are not of common occurrence in *cortezi*. Some of the crosses (Table 1) were among fish that represent a pure line from Arroyo Palitla while others are hybrids between Arroyo Palitla and strain 38 (Rio Axtla). Sex ratios for strain 38 are listed in Table 3. Of all pedigrees, only two (Table 1) showed a sex ratio that deviated significantly (at the 0.05 level) from unity (ped. 1800: 10 ♀♀ —26 ♂♂, $0.01 < P < 0.02$; ped. 2471: 20 ♀♀ —8 ♂♂, $0.02 < P < 0.05$).

Summary

The inheritance of four melanophore patterns was studied in the teleost *Xiphophorus montezumae cortezi*. Three of them, At (atromaculatus), Cam (carbomaculatus), and Sc (spotted caudal) are composed of macromelanophores, and the fourth one, Cb (caudal blot), of micromelanophores. The patterns are controlled by four loci that are not linked and not associated with sex. No abnormal sex ratios were obtained. At, Cam, and Cb are dominant, but *Sc* exhibits incomplete penetrance. In approximately 22 percent of the *Sc* fish of an inbred laboratory stock, the gene does not manifest itself; in hybrids between this stock and wild fish, the penetrance of Sc is only 30 percent. The frequency of the *Sc* factor in the population of the Rio Axtla has been estimated to be about 59 percent. Within the inbred stock, the expression of Sc may vary from a small elongate streak in the caudal fin to large melanomas that eventually destroy the fin. The melanoma may spread into the caudal peduncle. No fish with melanoma have been seen in preserved collections of *X. m. cortezi* or in hybrids between the inbred stock and wild fish. All the major populations studied are polymorphic for the four patterns, although there may be significant differences in their frequencies. The situation in *X. m. cortezi* where the macromelanophore patterns are controlled by three unlinked loci contrasts with the one present in *X. maculatus* and *X. variatus* where the patterns are controlled by the same gene or supergene.

Acknowledgments

The collections at the University of Michigan, Museum of Zoology, were examined through the courtesy of Dr. R. M. Bailey and Dr. R. R. Miller. Figures 2 to 8 were prepared by Mrs. Joyce Presseau. The research in the Genetics Laboratory is supported in part by grant CA 06665 of the National Cancer Institute, U.S. Public Health Service. All are gratefully acknowledged.

Literature Cited

Anders, A., and F. Anders

1963. Genetisch bedingte XX- und XY- ♀♀ und XY- und YY-♂♂ beim wilden *Platypoecilus maculatus* aus Mexico. Z. Vererbungsl., 94:1-18.

Anders, F., and K. Klinke

1965. Untersuchungen über die erbbedingte Aminosaürenkonzentration, Farbgenmanifestation und Tumorbildung bei lebendgebärenden Zahnkarpfen (Poeciliidae). Z. Vererbungsl., 96:49-65.

Atz, J. W.

1962. Effects of hybridization on pigmentation in fishes of the genus *Xiphophorus*. Zoologica 47:153-181.

Atz, J. W., K. D. Kallman, and R. F. Nigrelli

1963. Position effect as a factor in the production of melanosis and melanoma in the fish *Xiphophorus*. Proc. XVI Int. Congr. Zool., Washington 2:206.

Breider, H., and H. Mombour

1949. Das Farbgen *"Nigra-caudal"* (*Nc*) des *Xiphophorus montezumae*. Wschr. Aquar. Terr. Kd. 43 (11):309-313.

Clark, E., L. R. Aronson, and M. Gordon

1954. Mating behavior patterns in two sympatric species of xiphophorin fishes: their inheritance and significance in sexual isolation. Bull. Am. Mus. Nat. Hist. 103 (2): 135-226.

Darnell, R. M.

1962. Fishes of the Rio Tamesi and related coastal lagoons in east-central Mexico. Publ. Inst. Mar. Sci. Univ. Tex. 8:299-365.

Franck, D.

1964. Vergleichende Verhaltenstudien an lebendgebärenden Zahnkarpfen der Gattung *Xiphophorus*. Zool. Jb. Physiol. Bd. 71: 117-170.

1970. Verhaltensgenetische Untersuchungen an Artbastarden der Gattung *Xiphophorus* (Pisces). Z. f. Tierpsychol. 27:1-34.

Friedman, B., and M. Gordon

1934. Chromosome numbers in xiphophorin fishes. Am. Nat. 68:446-455.

Dzwillo, M., und C. D. Zander

1967. Geschlechtsbestimmung und Geschlechtsumstimmung bei Zahnkarpfen (Pisces). Mitt. Hamburg. Zool. Mus. Inst. 64: 147-162.

Gordon, M.

1940. Gene frequencies and parallel variations in natural populations of seven geographical species of Mexican fresh-water fishes. Genetics 25:118 (abstract).

1950. Fishes as laboratory animals. *In* the care and breeding of laboratory animals. E. Farris (editor). John Wiley and Sons, New York, pp. 345-449.

1951. Genetic and correlated studies of normal and atypical pigment cell growth. Growth, Symposium 10:153-219.

1952. Sex determination in *Xiphophorus* (*Platypoecilus*) *maculatus*. III. Differentiation of gonads in platyfish from broods having a sex ratio of three females to one male. Zoologica 37:91-100.

1956. Swordtails, the care and breeding of swordtails. T. F. H. Publ., Inc., Jersey City, N.J. 24 pp.

1957. Physiological genetics of fishes. *In* the physiology of fishes, Academic Press, vol. 2:431-501.

1958. Genetic and developmental differences between two morphologically similar pigment cells with reference to melanoma. Anat. Rec. 132:446 (abstract).

Gordon, M., and D. E. Rosen

1951. Genetics of species differences in the morphology of the male genitalia of xiphophorin fishes. Bull. Am. Mus. Nat. Hist. 95:409-465.

Kallman, K. D.

1965. Genetics and geography of sex determination in the poeciliid fish, *Xiphophorus maculatus*. Zoologica 50:151-190.

1968. Evidence for the existence of transformer genes for sex in the teleost *Xiphophorus maculatus*. Genetics 60:811-828.

1970a. Sex determination and the restriction of sex-linked pigment patterns to the X and Y chromosomes in populations of a poeciliid fish, *Xiphophorus maculatus*, from the Belize and Sibun Rivers of British Honduras. Zoologica 55:1-16.

1970b. Different genetic basis of identical pigment patterns in the teleost, *Xiphophorus maculatus*. Copeia, No. 3, 472-487.

Kallman, K. D., and J. W. Atz

1966. Gene and chromosome homology in fishes of the genus *Xiphophorus*. Zoologica 51: 107-135.

Kallman, K. D., and M. P. Schreibman

1971. The origin and possible genetic control of new, stable pigment patterns in the poeciliid fish, *Xiphophorus maculatus*. J. Exp. Zool., 176:147-168.

Kosswig, C.

1935. Die Kreuzung zweier XX-bzw. XY-Geschlechter miteinander und der Ersatz eines Y-Chromosoms einer Art durch das X-Chromosom einer anderen. Züchter 7: 40-48.

1936. Kleinere Mitteilungen über Art- und Gattungsbastarde von Zahnkarpfen. Zool. Anz. 114:195-206.

1959. Beiträge zur genetischen Analyse xiphophoriner Zahnkarpfen. Biol. Zentralblatt. 78:711-718.

1964. Polygenic sex determination. Experientia 20:1-10.

Kosswig, C., and M. Oktay

1955. Die Geschlechtsbestimmung bei den Xiphophorini (Neue Tatsachen und neue Deutungen). Istanbul Univ. Fen Fak. Hidrob., ser. B, 2:133-156.

Lueken, W., and W. Foerster

1969. Chromosomenuntersuchungen bei Fischen mit einer vereinfachten Zellkulturtechnik. Zool. Anz. 183:168-176.

MacIntyre, P. A.

1961. Crossing over within the macromelanophore gene in the platyfish, *Xiphophorus maculatus*. Amer. Nat. 95:323-324.

Peters, G.

1964. Vergleichende Untersuchungen an drei Subspecies von *Xiphophorus helleri* (Heckel) (Pisces). Z. zool. Syst. Evolutionsforschung 2:185-271.

Rosen, D. E.

1960. Middle-American poeciliid fishes of the genus *Xiphophorus*, Bull. Florida State Mus., Biol. Sci. 5:57-242.

Rosen, D. E., and K. D. Kallman

1969 A new fish of the genus *Xiphophorus* from Guatemala, with remarks on the taxonomy of endemic forms. Am. Mus. Novitates, no. 2379, 29 pp.

Schröder, J. H.

1964. Genetische Untersuchungen an domestizierten Stämmen der Gattung *Mollienesia* (Poeciliidae). Zool. Beitr. 10:369-463.

Sengün, A.

1949. Beitrage zur Kenntnis der erblichen Bedingtheit von Formunterschieden der Gonopodien lebendgebärender Zahnkarpfen. Istanbul Univ. Fen Fak. Mecm., B. 15:110-113.

Stöwahse, I., und W. Villwock

1949. Beiträge zur Kenntnis der erblichen lichen Differenzierung viviparer Zahnkarpfen (Pisces, Poeciliidae). I. *Xiphophorus*-Arten (1. Teil). Abh. Verh. Naturw. Ver. Hamburg, N. F. 13:31-118.

Zander, C. D.

1962. Untersuchungen über einen artrennenden Mechanismus bei lebendgebärenden Zahnkarpfen aus der Tribus Xiphophorini. Mitt. Hamburg. Zool. Mus. Inst. 60: 205-264.

1965. Die Geschlechtsbestimmung bei *Xiphophorus montezumae cortezi* Rosen (Pisces). Z. Vererbungsl. 96:128-141.

1967 Ökologische und morphologische Beiträge zur Systematik und geographischen Verbreitung der Gattung *Xiphophorus* (Pisces). Mitt. Hamburg. Zool. Mus. Inst. 64:87-125.

1968. Über die Vererbung von Y-gebundenen Farbgenen des *Xiphophorus pygmaeus nigrensis* Rosen (Pisces). Molec. Gen. Genetics 101:29-42.

1969. Über die Entstehung und Veränderung von Farbmustern in der Gattung *Xiphophorus* (Pisces). Mitt. Hamburg. Zool. Mus. Inst. 66:241-271.